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The geological record of protists begins well before the Ediacaran and Cambrian diversification of animals, but the antiquity of that history, its reliability as a chronicle of evolution, and the causal inferences that can be drawn from it remain subjects of debate. Well-preserved protists are known from a relatively small number of Proterozoic formations, but taphonomic considerations suggest that they capture at least broad aspects of early eukaryotic evolution. A modest diversity of problematic, possibly stem group protists occurs in ca. 1800-1300 million year old (Ma) rocks. 1300-720 Ma fossils document the divergence of major eukaryotic clades, but only with the Ediacaran-Cambrian radiation of animals did diversity increase within most clades with fossilizable members. While taxonomic placement of many Proterozoic eukaryotes may be arguable, the presence of characters used for that placement is not. Focus on character evolution permits inferences about the innovations in cell biology and development that underpin the taxonomic and morphological diversification of eukaryotic organisms.

Keywords: Eukaryote; fossil; Proterozoic; evolution

1. INTRODUCTION

In *The Origin of Species*, Charles Darwin (1859) famously fretted over the complexity of the oldest known animal fossils. To account for the stratigraphic pattern he observed, Darwin postulated a long prior history of metazoan evolution, during which the morphological complexity and diversity displayed by trilobites and other Cambrian animals slowly accumulated. The absence of confirming fossils was ascribed to massive failure of the pre-Cambrian stratigraphic record.

Today, we recognize a relative abundance of Proterozoic sedimentary rocks, distributed globally. Paleontologists have also documented Proterozoic animal fossils, but only from the last 30-40 million years (Ma) of the eon (Xiao & Knoll 2000; Narbonne 2005). On the other hand, insights from cell biology, molecular phylogeny, and developmental genetics have not only positioned animals within the greater diversity of eukaryotic organisms, but shown that the defining traits of animal life are built on a foundation of antecedent molecular features (e.g., Maynard Smith & Szathmáry 1995; King 2004).

The view that animal evolution was made possible, at least in part, by prior innovations in eukaryotic genetics and cell biology requires that eukaryotic life diversified before the advent of metazoans. Opinions on the antiquity of the Eucarya range widely (e.g., Brocks et al. 1999 *vs*. Cavalier-Smith 2002a), but molecular clock estimates commonly suggest an earlier Proterozoic origin and later Proterozoic diversification of the clade (Doolittle et al. 1996; Yoon et al. 2004, Douzery et al. 2004; in contrast, Hedges et al. 2004 proposed Paleoproterozoic origin *and* early crown group divergences). Such conjectures obviously make predictions about what paleontologists should see in Proterozoic sedimentary rocks. In

this paper, we review the early fossil record of eukaryotic organisms and use it to explore the cellular and functional evolution of protists in Proterozoic oceans.

2. AN EARLY EUKARYOTE

How do we recognize ancient fossils as eukaryotic? A concrete example serves to address this question. *Shuiyousphaeridium macroreticulatum* is known from a large population of microfossils preserved in coastal marine shales of the Ruyang Group, northern China (Yan & Zhu 1992; Xiao et al. 1997). The fossils are spheroidal organic vesicles characterized by a reticulated surface and numerous regularly spaced cylindrical processes that flare outward (ca. 80 processes are visible around the periphery of a single specimen; Fig. 1a,b). Vesicles range in diameter from 50 to 300 μm (mean, 148 μm; standard deviation, 38 μm); processes are hollow, 10-15 μm long, and 2-3 μm wide. SEM examination shows that the vesicle's outer surface is covered with ridges that delimit polygonal fields ca. 2 μm across (Fig. 1 c,f). Inner wall surfaces show the same ornamentation, but in reverse relief – fields are visible as closely packed, beveled hexagonal plates (Javaux et al. 2004; Fig. 1e). TEM images further show that the ca. 1.5 μm wall is multilayered, with the electron-dense, homogeneous layer of organic plates lying above a thin electron-tenuous layer (Javaux et al. 2004; Fig. 1d).

Prokaryotes can be large, they can have processes, and they can have preservable walls. But we know of no prokaryote that combines these three characters, and none that exhibits the complexity of form that light microscopy, SEM, and TEM document in *Shuiyousphaeridium*. Many eukaryotes do exhibit these features in combination; therefore, we believe the most straightforward interpretation of these fossils is that they were made by a eukaryotic organism. According to Cavalier-Smith (2002a, p.37), "cysts with spines or

reticulate surface sculpturing would probably have required both an endomembrane system and a cytoskeleton, the most fundamental features of the eukaryotic cell, for their construction." *Shuiyousphaeridium* clearly fits this description.

Phylogenetic conjectures for *Shuiyousphaeridium* range from dinoflagellates (Meng et al. 2004) to possible fungi (Butterfield 2004), but the fossils provide little support for any specific attribution. The population could represent either a stem or crown group eukaryote.

Radiometric dates indicate that Ruyang deposition occurred after 1600 million years ago (Ma) but before 1000 Ma. Carbon isotopic stratigraphy, in turn, suggests an age greater than 1250 Ma (Xiao et al. 1997). Thus, *Shuiyousphaeridium* was a Mesoproterozoic protist.

3. PALEONTOLOGICAL ESTIMATES OF EUKARYOTIC ANTIQUITY

Other fossils corroborate the observation that eukaryotes lived in early Mesoproterozoic oceans. Microfossils in the Ruyang Group include a second process-bearing taxon, *Tappania plana* (Yin 1997; Fig. 2a). In contrast to *Shuiyousphaeridium, Tappania* displays broadly spheroidal vesicles of variable size (20 to 160 μm) that bear 0 to 20 closed, heteromorphic processes distributed asymmetrically on the vesicle surface (Yin 1997; Javaux et al. 2001). Processes vary in length from 25 to 60 μm and uncommonly branch. A single specimen shows what may be a septum between vesicle and process (Butterfield 2005a), but, in general, processes communicate freely with the vesicle interior. Vesicles may also exhibit up to three bulbous extensions, suggestive of budding. In addition to their Chinese occurrence, *Tappania* populations have been found in reliably dated (U-Pb zircon age of 1492+/-3 Ma for a subtending ash bed) shales of the Roper Group, northern Australia (Javaux

et al. 2001), and in Mesoproterozoic successions from India (Prasad & Asher 2001) and Russia (Nagovitsin 2001).

Its irregular morphology and asymmetric distribution of processes suggest that *Tappania* was an actively growing vegetative cell or germinating structure rather than a metabolically inert spore (Javaux et al. 2001). As in the case of *Shuiyousphaeridium*, *Tappania*'s combination of large size, preservable walls, complex processes, and possible budding structures finds no matches among known Bacteria or Archaea. [Readers interested in the proposal that *Tappania* was an actinomycete similar in overall structure to extant *Kibdelosporangium* (a soil-dwelling, hyphal bacterium that synthesizes no decay-resistant wall polymers) should consult Shearer et al. (1989).] Butterfield (2005a) has proposed that *Tappania plana* was fungal, based on putative similarities to complex Neoproterozoic microfossils from Arctic Canada. Fungal affinity is possible, but given the limited number of systematically informative characters, we prefer to view this fossil as problematic (and distinct from the Neoproterozoic population to which it has been compared). Regardless of phylogenetic interpretation, however, we agree with Butterfield (2005a) that *Tappania* was a eukaryote with a complex cytoskeleton, probably preserved in an actively growing phase of its life cycle, and at least plausibly heterotrophic.

Other protists in the Roper assemblage include *Valeria lophostriata* (Jankauskas 1989; Xiao et al. 1997; Javaux et al. 2003; Fig. 2d,e) and *Satka favosa* (Jankauskas 1989; Javaux et al. 2003; Fig. 2c,f), distinguished by surface ornamentation of parallel ridges uniformly spaced on the internal surface of the vesicle and tessellated polygonal plates up to 15 microns across, respectively. Roper microfossils additionally include three leiosphaerids (simple organic-walled spheres) with distinctively heterogeneous wall ultrastructures, as observed under TEM

(Fig. 2g,h). Once again, such wall structure is common among extant protists that make preservable walls of comparable size and morphology, but distinct from the most likely prokaryotic candidates, envelope-forming cyanobacteria (Javaux et al. 2004).

Macroscopic compressions, impressions, and casts occur globally in rocks of comparable age. Ca. 1450 Ma shales of the Helena Formation, Montana contain a variety of carbonaceous compressions up to several cm long (Walter et al. 1976). Of these, coiled fossils assigned to *Grypania spiralis* are most confidently interpreted as eukaryotic. (Most other forms could be fortuitously shaped fragments of microbial mats.) *Grypania* fossils are narrow ribbons, originally cylindrical, up to 13 mm long and 2 mm wide, that commonly form a regular coil up to 24 mm across (Walter et al 1990; Fig. 2i). Indian populations illustrated by Kumar (1995) preserve a distinct mm-scale annulation that may reflect underlying cell structure. These fossils are very likely of eukaryotic origin, although phylogenetic relationships are not well constrained. Well preserved *Grypania* populations occur, as well, in Mesoproterozoic rocks from China (Walter et al. 1990), and Paleoproterozoic (ca. 1850 Ma) populations have been reported from Canada (Han & Runnegar 1992). Samuelsson and Butterfield (2001) regard these latter specimens as possible composites of much smaller prokaryotic filaments, but the pronounced morphological regularity of specimens within a large population examined by one of us (AHK) convinces us that the Canadian *Grypania* was an organism, not a colony or composite.

The other early Mesoproterozoic macrofossil with a pursuable claim to eukaryotic status is *Horodyskia moniliformis*, known as casts and molds in sandstones from Montana and Western Australia (Horodyski 1982a; Grey & Williams 1990; Yochelson & Fedonkin 2000; Fig. 2b). *Horodyskia* consisted of 1-4 mm spheroidal (less commonly ovoid, rectangular, or

conoidal) bodies connected by thin cylindrical strings to form uniseriate, pearl-necklace-like structures up to 10 cm long. Grey and Williams (1990) drew structural analogies to articulated seaweeds, whereas Yochelson and Fedonkin (2000) favored comparisons with animals. In fact, archeal-bacterial consortia in modern sulfur springs form mm-scale strings of beads (Rudolf et al. 2001), although it remains an open question whether such features would form or preserve in the environments inferred for *Horodyskia* fossils. We interpret *Horodyskia* as a problematic macrofossil whose eukaryotic affinities are probable, but not beyond debate.

As discussed by Summons in this issue (Summons 2005), preserved steranes independently suggest that eukaryotic organisms inhabited mid-Proterozoic oceans. If eukaryotes diverged only 700-800 Ma (Cavalier-Smith 2002a), then all putatively eukaryotic fossils and biomarkers in older rocks must be misinterpreted, and numerous mid-Proterozoic prokaryotes must have possessed attributes that were subsequently lost and re-evolved convergently by eukaryotes. If any one of these records is correctly interpreted, eukaryotes existed in mid-Proterozoic oceans.

Other fossils in mid-Proterozoic rocks could be eukaryotic, but their affinities are less clear. Large (>50 μm) spheroidal microfossils are widely distributed in Mesoproterozoic shales. As noted above, a few of these are known to have walls with complex ultrastructure of types best known from eukaryotes, but most have unknown wall structure and could, in principle, be preserved envelopes of colonial cyanobacteria (Knoll 1996). Similarly, longitudinally striated carbonaceous tubes up to 150 μm in diameter and more than a millimeter long found in shales of the Roper Group could be eukaryotic, but this interpretation remains tentative.

Can we extend the eukaryotic fossil record backward into the Paleoproterozoic or beyond? The oldest acritarchs (a collective term applied by paleontologists to closed, organic walled microfossils of uncertain systematic affinities) with regular ornamentation are populations of the corduroy-like *Valeria* preserved in shales of the Mallapunyah Formation, northern Australia, closely constrained by radiometric ages to about 1650 Ma (Javaux et al. 2004), and in the Changcheng Group, northern China, constrained to be >1683+/-67 Ma and <1780+/-20 Ma (Yan & Liu 1993; Li et al.1995; Wan et al. 2003). Changcheng assemblages also include large (up to 237 μm) unornamented acritarchs, some of which display regular medial splits, similar to those formed during excystment of younger protists (Yan & Liu 1993). The splits are both common and regularly equatorial, favoring a eukaryotic interpretation. Cyanobacterial envelopes can split in regular patterns (Waterbury & Stanier 1978), however; so the medially split leiosphaerids in Changcheng rocks are conservatively interpreted as possibly but not unambiguously eukaryotic. Comparable uncertainty attends uniseriate filaments up to about 100 μm wide, also found in Changcheng shales (Yan & Liu 1993).

Carbonaceous compressions in late Paleoproterozoic shales from China have been interpreted as seaweeds, with specimens from the 1600-1700 Ma Tuanshanzi Formation specifically attributed to the Phaeophyta (Zhu & Chen 1995). Examination of Tuanshanzi structures in outcrop by one of us (AHK) suggests that the features in question can alternatively be interpreted as rare, fortuitously shaped fragments deposited among many irregular mat shards. Lamb, Awramik, and Zhu (2005) have drawn similar conclusion about macroscopic compressions in the older Changzhougou Formation. Steranes in 2780 Ma shales from Western Australia have been interpreted as evidence that stem eukaryotes diverged early

in Earth history (Brocks et al. 1999), but a general paucity of well-preserved microfossils precludes independent morphological inferences about eukaryotic biology prior to about 1800 Ma.

In summary, late Paleoproterozoic and early Mesoproterozoic rocks preserve evidence for a moderate diversity of preservable eukaryotic organisms. This evidence includes cell walls without surface ornament (but with complex ultrastructure) and walls with regularly distributed surface ornamentation, with asymmetrically arranged processes that appear to reflect active cell growth, and with numerous symmetrically arranged processes. Collectively, these fossils suggest that eukaryotes not only existed in mid-Proterozoic oceans, but possessed flexible membranes and cytoskeletons capable of directing cell remodeling and surface morphology (Javaux et al. 2001; see below).

4. DISPARITY AND DIVERSITY AMONG LATER PROTEROZOIC EUKARYOTES

(a) Phylogeny

Phylogenetic attribution and diversity history are enduring issues in paleontology, no less so in Proterozoic than in Phanerozoic research. Paleobiological studies of plant and animal evolution show that while reliable phylogenetic placement of crown group fossils can be straightforward, interpretation of early diverging stem groups is not. Fossils can frustratingly display only a small subset of the characters that collectively distinguish crown groups and, even worse, can exhibit character combinations not observed in any extant clade. The problem is all the more difficult for early eukaryotes, as only selected features (mostly cell walls) are candidates for preservation, and preserved fossils can be undiagnostically simple.

Many, perhaps most, preserved Proterozoic protists cannot be assigned with confidence to any specific branch of the eukaryotic tree (to the extent that we know it). A few, however, preserve diagnostic features of life cycle and morphology that support reasonable systematic interpretation. (The formation of preservable parts is, itself, a character of interest, as many protists synthesize no preservable walls or cysts during their life cycle.) Butterfield (2000) has marshaled a strong case for the close phylogenetic relationship of the fossil *Bangiomorpha pubescens* (Fig. 3c) to bangiophyte red algae. *Bangiomorpha* bears a superficial resemblance to uniseriate filamentous cyanobacteria, but as Butterfield (2000) points out, it differs in a number of key characters. *Bangiomorpha* filaments have cellularly differentiated holdfasts and zones of discoidal cells that expand and divide radially in several planes to produce distinctive wedge-shaped cells. Such features are unknown in cyanobacteria, but occur together along with other characters displayed by *Bangiomorpha* in extant bangiophyte red algae (Butterfield et al. 1990; Butterfield 2000). Moreover, taphonomic features displayed by *Bangiomorpha* are similar to those of other early eukaryotes, but different from those of filamentous cyanobacteria known from the Proterozoic fossil record. *Bangiomorpha* displays three-dimensionally competent preservation of outer and inner walls, with no cytoplasmic preservation, whereas cyanobacterial filaments with sheaths generally show competent three-dimensional preservation of the sheath, but partial or complete collapse of the cells inside (Bartley 1996). Published radiometric dates constrain *Bangiomorpha* only to the interval 1267+/-2 to 723+/-3 Ma, but an unpublished Pb-Pb date of 1198+/-24 Ma and physical stratigraphic relationships strongly suggest that the fossils' age lies close to the lower radiometric boundary (Butterfield 2000).

Latest Mesoproterozoic (>1005+/-4 Ma; Rainbird et al. 1998) microfossils from the Lakhanda Group, Siberia, contain several additional populations of coenocytic to multicellular filaments whose morphologies and dimensions suggest eukaryotic origin (Herman 1990; Fig. 3f). Principal among these are fossils assigned to *Palaoevaucheria clavata* and other form taxa that appear to preserve vegetative and reproductive phases of a heterokont protist comparable to the extant xanthophyte alga *Vaucheria* (Jankauskas 1989; Herman 1990). *Vaucheria*-like populations preserving several life cycle stages also occur in the 750-800 Ma Svanbergfjellet Formation, Spitsbergen (Butterfield 2004). Latest Meosoproterozoic and early Neoproterozoic acritarchs (Fig. 3h) continue the record of moderate diversity established earlier, although some taxa characteristic of these younger assemblages have not, to date, been found in older rocks (Knoll 1996).

By 750-800 Ma, the most diverse fossil assemblages contain an increased diversity of acritarch and other protistan morphotypes (e.g., Butterfield et al. 1994; Butterfield and Rainbird 1998), including small branched structures interpreted as siphonocladalean green algae (Butterfield et al. 1994); vase-shaped structures interpreted as both filose and lobose testate amoebae – and, therefore, as both amoebozoan and cercozoan protists (Porter et al. 2003; Fig. 3i); remarkable spheroidal fossils from which numerous anastomosing cellular filaments arise, interpreted as possible fungi (Butterfield 2005a; Fig. 3a,b); and a moderate diversity of other colonial to multicellular eukaryotes with less certain systematic affinities (Butterfield et al. 1994; Butterfield 2005b). Collectively, carefully studied microfossil assemblages support the hypothesis that the later Mesoproterozoic and early Neoproterozoic was a time of major clade divergence within the Eucarya, although diversity within most major clades remained relatively low (see Porter 2004 for a recent review).

(b) Diversity

A decade ago, several labs published attempts to divine diversity history from the Proterozoic acritarch record (Zang & Walter 1992; Schopf 1992; Knoll 1994; Vidal & Moczydlowska 1997). Those that included all available data agreed in depicting a eukaryotic record with modest Mesoproterozoic diversity, higher but still relatively low diversity in the Neoproterozoic, an early Ediacaran (ca. 580-560 Ma) spike in taxonomic richness followed by low late Ediacaran taxonomic diversity, and, then, marked Cambrian diversification. These studies have been criticized on several counts, including poor taxonomy, incomplete sampling, and misinterpretation of inferred ecology, especially for acritarchs (Butterfield 2005a,b). Most early analyses accepted that Proterozoic acritarchs were, like those in Paleozoic rocks, largely the reproductive cysts of planktonic algae. We agree with Butterfield (2005a,b), however, that Proterozoic acritarch assemblages include, and in some cases may be dominated by, vegetative remains of organisms that were heterotrophic rather than photosynthetic, and benthic rather than planktonic. Taxonomic issues are being resolved by continuing research, bolstering our belief that meaningful if qualitative comparisons can be made among assemblages and stratigraphic intervals.

In studies of Phanerozoic diversity, the problem of sampling in compilations of total diversity has been addressed in part by comparison with records of taxonomic richness for individual assemblages, broad proxies for community diversity that are not subject to many of the biases thought to influence global samples (Bambach 1977). Fig. 4 (see also Appendix) shows a comparison of eukaryotic diversity for a selection of well preserved and well studied Proterozoic to Early Cambrian fossil assemblages. Consistent with discussions in the preceding section, we have included all non-metazoan fossils whose organization,

ornamentation, and/or ultrastructure suggest a likely eukaryotic origin. The most problematic fossils included in our compilation are leiosphaerids, spheroidal acritarchs without distinguishing ornament. In some cases (e.g., Butterfield et al. 1994; Javaux et al. 2004), TEM observations support a eukaryotic origin for these fossils, but ultrastructural data are lacking for most Proterozoic leiosphaerids. By including only leiosphaerids with a diameter greater than 50 μm, we have likely excluded smaller protistan fossils and may, as well, have included cyanobacterial envelopes. Our compilation segregates leiosphaerids from other presumable eukaryotes, enabling readers to track different fossil morphotypes through time.

From these assemblages, we infer the following:

1. In late Paleoproterozoic to early Mesoproterozoic rocks (ca. 1600-1300 Ma), eukaryotic biology is recorded by a modest diversity of macroscopic fossils and preserved cell walls, including forms with complex ultrastructure, regular ornamentation, and/or cylindrical processes. These fossils may but need not include crown group eukaryotes.

2. Late Mesoproterozoic and early Neoproterozoic (ca. 1300-720 Ma) assemblages continue the record of modest acritarch diversity, although many taxa found in rocks of this age differ from those in earlier assemblages. Unornamented and ornamented sphaeromorphs, some showing evidence of binary division or budding, are common. Acritarchs with asymmetrically placed processes (in some cases reflecting actively growing cells; Knoll et al. 1991; Butterfield et al. 1994; Butterfield 2004) form a taxonomically and numerically subordinate part of assemblages, and acritarchs with symmetrically distributed processes are uncommon. Read literally, the published record suggests that acritarch diversity was a bit higher in the later part of this interval (ca. 800-720 Ma) than in the earlier part (Fig. 4b). Certainly, the later record includes a greater diversity of non-acritarchous eukaryotes,

especially vase-shaped protists and microscopic multicellular protists (Fig. 4d). Fossils from this interval include the earliest recognizable representatives of extant eukaryotic clades, including red algae, green algae, heterokonts, amoebozoans, cercozoans, and possibly fungi (Porter 2004). Macroscopic compressions document a small diversity of cm-scale blades and closed tubes (Du & Tian 1985; Hofmann 1992; Fig. 4c).

The curtain drops on these assemblages with the onset of Sturtian glaciation, and it rises again on a substantially different biota only after the Marinoan glaciation ca. 632 Ma. At least some earlier Neoproterozoic morphotypes survived the Sturtian ice age (Allison & Awramik 1989; redated by Kaufman et al. 1992), but only a few microfossil assemblages unambiguously document marine life between the major glaciations. Whether this reflects persistently unfavorable environments (e.g., James et al. 2004) or bad luck in sampling is, at present, unclear.

3. Early Ediacaran (632 to ca. 580-550 Ma) assemblages display a notable increase in observable diversity, and the composition of these assemblages also shifted markedly -- for the first time, populations with symmetrically distributed processes dominate acritarch biotas (Zang & Walter 1992; Zhang et al. 1998; Moczydlowska et al. 1993; Grey 2005; Fig. 4a,b). Macroscopic compressions in coeval or slightly younger rocks similarly record a much higher diversity of form, including a variety of seaweeds and possible animals (Steiner 1994; Xiao et al. 2002; Fig. 4c). Macrosocopic algae include the first dichotomously branching thalli (Fig. 3d), as well as anisotomously branching forms. Animals are independently known from Ediacaran phosphorites, carbonates and sandstones (Xiao & Knoll 2000; Grotzinger et al. 2000; Narbonne 2005). Interestingly, late Ediacaran (ca. 550-542 Ma) acritarch assemblages known to date display none of the acanthomorphic diversity so obvious in preceding deposits

(Fig. 4a). Unornamented spheroids, commonly abundant and including forms several hundred microns in diameter, dominate microfossil assemblages (Germs et al. 1986; Jankauskas 1989; Burzin et al. 1997).

4. Cambrian and Ordovician rocks record renewed diversification of ornamented and symmetrical process-bearing acritarchs – now, we believe, properly ascribed to cyst-forming algae (and, perhaps, heterotrophic dinoflagellates). This two-step increase parallels the Cambrian and Ordovician radiations of marine animals (Knoll 1994; Vidal & Moczydlowska 1997; Fig. 4a,b), as well as the diversification of skeletal protists, including both foraminiferans and radiolarians (see references in Knoll 2003a).

How reliable is this record as a chronicle of evolution? This question deserves fuller treatment than can be provided here, but the short answer is, "Reasonably, with a few caveats." Acritarchs comprise the most abundant and widely distributed record of Proterozoic protists, and the assemblages noted for the five intervals outlined above each occur in at least four different basins on multiple continents, preserved as both compressions in shale and silica permineralizations (and, in Ediacaran successions, phosphorite). The basic trends outlined for acritarch morphology and diversity appear to be broadly predictive and, therefore, reflective of evolutionary history. The same is true of macroscopic fossils. Early Mesoproterozoic assemblages characterized by *Grypania* and *Horodyskia*, an earlier Neoproterozoic assemblage dominated by the morphotaxa *Tawuia* and *Longfengshania*, diverse Ediacaran macroalgal assemblages, and Ediacaran to Cambrian animal assemblages occur in stratigraphically predictable fashion. Moreover, some of the macroalgae documented in early Ediacaran shales also left a conspicuous signature in sandstones, decreasing the probability that older records would be missed. For example, the cm-scale, originally fluid-filled

macrosphere *Beltinellaformis* is preserved in Ediacaran but not much older sandstones as casts and molds that commonly cover bedding planes (Xiao et al. 2002). Similarly, concentric grooves made by basally attached, semi-rigid algae or animals are well known from Ediacaran but not older bedding surfaces (Jensen et al. 2002). We accept the large diversity increase observed in macroscopic compressions as evidence for early Ediacaran algal diversification.

A similar case can be made for the three-dimensionally preserved vase-shaped protists described from ca. 800-700 Ma rocks. Taxonomic diversity in this class of fossils is best gauged from exceptionally preserved assemblages in early diagenetic dolomite concretions within Grand Canyon shales (Porter et al. 2003), but because of the rigid nature of their tests, vase-shaped protists are commonly preserved as casts in mid-Neoproterozoic carbonates and cherts. Indeed, such casts can be among the most abundant fossils in these rocks (e.g., Knoll & Calder 1983), but they have not yet been reported from older successions.

The fossils that are most problematic in this regard -- frustratingly so, as they provide some of our best information on phylogeny -- are the multicellular fossils found as microscopic compressions in shales and, less commonly, as permineralizations in chert (Herman 1990; Butterfield 2000, 2004a, 2004b, 2005). The best argument we can muster here is that such microfossil complexity has been found repeatedly in late Mesoproterozoic and Neoproterozoic biotas but not in older assemblages that appear to be comparably well preserved. Once again, the record read literally may well provide a historical chronicle of early eukaryotic divergence, but we stress that the record as currently known provides minimum estimates of character evolution and clade divergence that may well change with continuing discovery.

5. A FUNCTIONAL APPROACH TO EARLY EUKARYOTIC FOSSILS

As summarized in the preceding section, Proterozoic protists included simple, unornamented unicells; morphologically complex and elaborately ornamented vesicles; clusters (colonies?) of uniform cells; uniseriate filaments, both branched and unbranched; and three-dimensionally complex multicellular organisms displaying cellular differentiation. For many of these fossils, phylogenetic placement is difficult because few characters ally fossil populations with extant clades. We can sidestep these issues by focusing on the characters themselves, and not on any implied phylogenetic affinity. For example, *Tappania* may or may not be related to fungi, but it inarguably displays an asymmetrical arrangement of processes. Similarly, one might debate the attribution of *Bangiomorpha* to the red algae, but it demonstrably exhibits cell differentiation. This suggests an independent, "taxonomy-free" avenue of inquiry focused on function, development, and the cell biological processes that underlie these features.

(a) Cell morphology and cytoskeletal complexity

As previously noted, populations of the Mesoproterozoic protist *Tappania* uniformly display processes on one side of the preserved vesicle but not the other, as well as distinctly and asymmetrically positioned bud-like emergences. The range of variation exhibited by these populations, in turn, strongly suggests that *Tappania* was able to modify cell shape during vegetative growth or zoospore germination (Javaux et al. 2001). Living cells with similar attributes establish polarity and modify cell shape via cytoskeletal organization (and reorganization). *Shuiyousphaeridium macroreticulatum* further documents the capacity of some Mesoproterozoic cells to construct elaborate processes at regular sites across the cell

wall, a function that must reflect localized secretion of wall materials and, therefore, intracellular mapping and molecular transport. Physical processes not necessarily related to cell biology can result in regular, self-organized structures (e.g. Li et al 2005), but the irregularities and apparent remodeling of cell shape exhibited by *Tappania* processes argue against formation purely by macromolecular self assembly. Other cell wall morphologies recorded by Proterozoic protists -- including equatorial arrangement of processes (*Germinosphaera fibrilla*; Butterfield et al. 1994; Butterfield 2005a) and unipolar localization of a large aperture (vase-shaped tests; Porter et al. 2003) – also reflect the capacity to localize structures to discrete positions in the cell, providing additional evidence for sophisticated eukaryotic cell organization well before the appearance of metazoans. Insofar as flexible membranes and a functioning cytoskeleton are fundamental to the eukaryotic condition (Cavalier-Smith 2002b), it is not surprising that the earliest fossils recognizable as protists should reflect such features of cell biology.

(b) What does filamentation tell us?

Filament construction requires that cell division occur consistently along a single axis or that cell growth proceed regularly at a point opposite (or otherwise specified) to the conjunction of two cells. Both processes require that the cell be able to map locations within itself relative to the cells to which it is attached (and not solely relative to environmental cues). Thus, filament formation requires both endogenous intracellular mapping to establish polarity and communication within and between adjacent cells. The cell polarization evident in asymmetrically distributed processes of *Tappania* need not reflect endogenous signaling, as

the spatially heterogeneous growth of its processes could, in principle, have been induced by an exogenous signal.

Like the vegetative expansion of *Tappania* processes, growth of multicellular filaments requires a means of molecular transport to specific locations – i.e., polarized transport. It is possible that materials used for differential growth moved by simple diffusion, and that localized growth in older Proterozoic cells reflects anabolic enzymes attached to the cell cortex. However, the ubiquity of the proteins used for intracellular transport argues for the antiquity of this process within the Eucarya -- the phylogenetic breadth of organisms known to contain genes coding for actin, myosin (Richards & Cavalier-Smith 2005), tubulin (Baldauf 2000), kinesin (Lawrence et al 2002), and dynein (Asai & Wilkes 2004) suggests the microtubule/kinesin/dynein and F-actin/myosin systems for intracellular transport evolved early in eukaryotic evolution.

Uniseriate filaments interpreted as eukaryotes occur in later Mesoproterozoic rocks (Herman 1990; Butterfield 2000) and may be represented by the late Paleoproterozoic (1780±20--1683±67 Ma) filament *Qanshania* (Han & Liu 1993). There is no reason to believe that any of the filamentous protists preserved in Proterozoic rocks were directly ancestral to animals. Rather, such fossils document the early evolution of a molecular capacity for simple multicellularity exploited through time by multiple clades.

Compared to simple filaments, branched structures allow more effective exploration for and exploitation of environmental resources. For example, fungi grow more densely in richer growth media (Ritz 1995) due to increased branching that allows more effective space filling and, therefore, increased absorptive capacity. Branched forms require a more sophisticated intracellular spatial organization because cellular locations must be mapped in a

way that is geometrically more complex than simply "opposite prior site of growth or division." Branched filaments evolved in the Eucarya no later than ca. 1000 Ma (Herman 1990) and were present in several clades by 800-720 Ma (Butterfield et al. 1994; Butterfield 2005a). The oldest branching structures of any type observed to date in Proterozoic protists are the bifurcating processes of nearly 1500 Ma *Tappania* (Javaux et al. 2001).

(c) Cellular differentiation

The question of cellular differentiation has long intrigued biologists. Even unicellular eukaryotes, such as *Saccharomyces cerevisiae*, undergo cellular differentiation, and the complex genetic interactions that bring about this differentiation are well studied (Alberts et al 2004). The late Mesoproterozoic red alga *Bangiomorpha* had at least three distinct cell types. *Palaeovaucheria* also exhibits cellular differentiation (Butterfield 2004), and complex multicellular structures found in ca. 820-780 Ma rocks from arctic Canada (Butterfield 2005a; Fig. 3a,b) appear to have had two distinct developmental programs, one for the large central cell and one for the filamentous mesh that surrounds it.

(d) Multicellularity in three dimensions

Three-dimensionality is prerequisite for tissue level organization. It can create an internal environment in which an outer layer of cells can protect interior cells from environmental challenges, and it also makes possible the mechanical support needed to construct large structures.

To build three-dimensionally organized structures that are mechanically stable (as opposed to loose tangles of filaments or consortia of cells), cells must be able to adhere to each

other via cell wall fusion or cell membrane adhesion. Indeed, cells must adhere to neighbors that are not the immediate products of a given division, requiring a sophisticated level of cell-cell communication that enables individual cells to accept adherence to their kin. Mesoproterozoic and early Neoproterozoic macrofossils, such as *Grypania* and *Longfengshania*, may reflect a molecular capacity for tissue formation, but in the absence of anatomical data, this is hard to demonstrate (or refute). The first clear example of three-dimensional organization and cell wall fusion is the complex "*Tappania*" fossils reported by Butterfield (2005a) from 820-780 Ma rocks in Canada (Fig. 3a,b). Diverse fossils in Ediacaran rocks document tissue-grade three-dimensionality in florideophyte red (Xiao et al. 2004) and other (Xiao et al. 2002) algae, discrete apical and intercalary meristems in diverse seaweeds (Xiao et al. 2001), and, of course, the complex ontogenies and astogenies of early macroscopic animals (Narbonne 2005).

(e) More cell-cell communication in the Proterozoic?

Populations of *Eosaccharomyces ramosus* from the >1005+/-4 Ma Lakhanda Suite, Siberia, formed a distinctive net-like pattern in which cells were oriented along anastomosing strands – not unlike cell arrangements in aggregating slime molds today (Herman 1990). Such population-scale pattern formation suggests a system of biochemically mediated behavior in which individual cells modified their movement and/or growth in response to molecular cues from conspecific neighbors (Knoll 1992). This, in turn, implies a ligand-receptor system. Of course, the inference that Proterozoic fossils had sexual life cycles, argued specifically for *Bangiomorpha* on morphological grounds (Butterfield 2000), implicitly accepts a system of communication and behavioral response among gametes. In *Eosaccharomyces*, however,

behavioral responses are preserved directly in the spatial distribution and orientation of cells within local populations.

(f) Relationship to observed evolutionary pattern

From the forgoing, one can draw two conclusions. First, eukaryotic fossils in Proterozoic rocks, long interpreted in terms of morphology and phylogeny, also permit inferences about the evolution of cell biology. Second, consistent with inferences drawn from comparative biology, fossils suggest that the cellular and molecular capacity for generating morphological diversity in protistan unicells and filaments evolved early in their evolutionary history. If correct, then other factors must be invoked to explain the long interval between the first appearance of eukaryotes in the geological record and their observed taxonomic radiation in latest Proterozoic to Cambrian oceans.

6. CONTROLS ON EARLY EUKARYOTIC DIVERSIFICATION

In principle, genetics, ecology, or environment could govern the pattern of protistan evolution inferred from Proterozoic fossils. Genetic facilitation is most difficult to assess, not least because the genetic determinants of morphology in most single-celled eukaryotes and algal thalli remain poorly understood. Nonetheless, observations reviewed in the preceding section suggest that the basic molecular mechanisms that control morphogenesis in unicellular and simple multicellular eukaryotes were in place fairly early in the Proterozoic Eon. Thus, without questioning the role of genetic innovation in the evolution of animals and complex, tissue-grade algae, it seems likely that early Ediacaran diversification of acritarchs and

macrophytes must reflect, at least in part, influences from ecology and/or environmental change.

Because eukaryotic phytoplankton and radiolarians diversified in step with marine invertebrates during the Cambrian and Ordovician periods, ecology can be justifiably invoked as a major driver of protistan evolution in the age of animals (Knoll 1994; Butterfield 1997). Butterfield (1997) proposed grazing by newly evolved mesozooplankton as a governing influence on the proliferation of ornamented and process-bearing acritarchs, although it must be remembered that the acritarch forms most common in Cambro-Ordovician rocks are cysts (Strother 1996), many of which may have rested on the sea floor like those of modern dinoflagellates (Dale 1983). Diversity increase and a pronounced coeval increase in rates of evolutionary turnover (Knoll 1994) could reflect the further influences of metazoan grazing and excretion on water mass heterogeneity at the appropriate spatial scale, or checks by grazers on the population growth of superior algal competitors. To the extent that nutrient depletion induces cyst differentiation in planktonic algae, increased patchiness of nutrients could also enhance the selective advantage of life cycles with resting stages -- imparting a spatial heterogeneity to marine phytoplankton that might, itself, facilitate diversification. Ecologists continue to debate the physical and biological controls on phytoplankton diversity in modern environments (e.g., Grover & Chrzanowski 2004), so it is not surprising that the nature of animal-protist interactions in Cambro-Ordovican seas remains incompletely understood. Argued in reverse, the absence of macroscopic grazers might explain the low diversity and long stratigraphic ranges of most Proterozoic acritarchs (Peterson & Butterfield 2005).

Where is the line of demarcation between these two states of the marine ecosystem? Peterson and Butterfield (2005) drew it at the beginning of early Ediacaran acritarch radiation, in the wake of global Marinoan glaciation. Post-Marinoan rocks contain the first acritarch assemblages dominated by forms with abundant, symmetrically arrayed spines, features interpreted by Peterson and Butterfield (2005) as mechanical defenses against predation by newly evolved benthic bilaterians. This explanation is plausible, although spiny Ediacaran acrtiarchs antedate the first geological records of motile bilaterians by as much as 25-50 million years, and the entire assemblage of spiny acritarchs disappears by the time that bilaterian trace fossils enter the record. The systematic relationships of early Ediacaran acritarchs remain unclear, but given their size, (in some cases) multicellular contents, and strong morphological similarity to egg hulls made by living animals (van Waveren & Marcus 1993), these distinctive fossils could well provide direct rather than exclusively indirect evidence for early animal diversification (see Yin et al. 2004). With less conviction, grazing arguments might also be applied to the early Ediacaran radiation of macroscopic seaweeds.

Whatever the nature of specific interactions, there can be little doubt that emerging animals influenced the continuing evolution of protists in late Neoproterozoic and Cambrian oceans. Does this obviate any role for environmental change? Of course not. Environmental and ecological facilitation are not mutually exclusive, and so ecological plausibility does not constitute evidence against environmental facilitation (*pace* Peterson & Butterfield 2005). Hypotheses of environmental influence may be correct or incorrect, but their tests lie in the precise stratigraphic resolution of geochemical and paleobiological data.

The dawning age of animals is associated with several environmental events of limited duration and one long term state change in the Earth system. Two global ice ages (and at least

one additional, regionally important glaciation; Halverson et al. 2005) and a brief but pronounced C-isotopic event coupled to transient shallow water anoxia near the Proterozoic-Cambrian boundary (Amthor et al. 2003) could each have influenced evolution by removing a large proportion of pre-existing eukaryotic biomass, creating in their wakes permissive ecologies in which novel (and not necessarily fitter) mutants generated by surviving populations could persist under low selective pressure, providing raw materials for evolutionary innovation (Knoll 2003b). We have only limited knowledge of life after the Sturtian (ca. 720-710 Ma) ice age, but there is evidence of evolutionary innovation in the wakes of both the Marinoan (ca. 650-635 Ma) ice age and the Proterozoic-Cambrian boundary event.

There is also widespread agreement that oxygen levels rose in the surface ocean and atmosphere during the Neoproterozoic Eon (e.g., Canfield & Teske 1995; Catling & Claire 2005; Holland 2005). Evidence from S isotopic fractionation (Shen et al. 2003), secular variation in S isotopes (Kah et al. 2004), and sulfate abundances in carbonate lattices (Gellatly & Lyons 2005) collectively indicate that sulfate levels in Mesoproterozoic oceans were lower than today's by about an order of magnitude, implying P_{O2} lower than at present. Fe speciation chemistry (Shen et al. 2003), Mo isotopes (Arnold et al. 2003), and biomarker profiles that document anoxygenic phototrophs in open waters (Brocks et al. 2005) independently suggest that sulfidic conditions were easily induced in the oxygen minimum zones (if not all deep waters) of Mesoproterozoic oceans.

The unresolved question is when oceans transited to a more modern state. A single sample from ca. 750 Ma black shales in the Grand Canyon exhibits the increased S-isotopic fractionation associated with oxygenic water columns (Canfield & Teske 1995), but marine

shales do not routinely preserve this biogeochemical signal until after 580 Ma (Hurtgen et al. 2004), suggesting a potentially close temporal relationship between the rise of oxygen and the appearance of both macroscopic animals and seaweeds. Rising oxygen might have influenced algal evolution in two ways – indirectly via the origin and diversification of large bilaterian animals and directly by the effects of redox change on nutrient status (Anbar & Knoll 2002). Unfortunately, no known geochemical process trips a recordable mineralogical or biogeochemical switch at P_{O2} levels that constitute physiological barriers to large size and motility in animals. Complicating the issue further, there is no reason to believe that oxygen levels trended monotonically through the Proterozoic Eon. The presence of iron formation in successions associated with Sturtian glaciation suggests to some (e.g., Canfield 1998; Holland 2005) that oxygen levels may have been especially low during times of Neoproterozoic climatic extremes.

In the end, *all* hypotheses to explain observed patterns of protistan evolution require better stratigraphic data: geochemical data that illuminate short term climatic and biogeochemical events as well as long term redox change, and fossil and biomarker data that document the first appearances of protists and ecologically important animals. Better data, in turn, will require improved models -- oceanographic, paleophysiological and ecological – to integrate paleobiological and paleoenvironmental observations. We predict that a mature understanding of protistan evolution will implicate genetics, ecology, *and* geophysical change rather than single any one of them out as a solitary driver.

7. CONCLUSIONS

Much has been learned about Proterozoic life in the oceans, but, clearly, much remains to be learned. Straightforward discovery played a principal role in the past two decades of

research, and it will likely continue as the main source of new understanding for some time to come. Particularly important will be the elucidation of well preserved assemblages from post-Sturtian but pre-Marinoan rocks and from Paleo-Mesoproterozoic successions.

New ways of looking at existing fossils will also loom large, however; TEM and microchemical techniques that can elaborate the physical and chemical composition of individual fossils will increasingly provide insights into phylogenetic relationships of early eukaryotes (e.g., Arouri et al. 1999; Talyzina 2000; Javaux et al. 2004; Marshall et al. 2005; Schopf & Kudryavtsev 2005). Finally, improved geochemical techniques for tracking the redox history of sea water, coupled with new and better insights into protistan genetics and ecology, will provide badly needed context for understanding the major changes recorded by eukaryotic fossils in Ediacaran and Early Cambrian rocks. The same questions that animated Proterozoic paleontological research a decade ago animate it now, but the data are richer and the answers are getting better. The same will undoubtedly be true a decade hence.

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Figure Captions

Figure 1. *Shuiyousphaeridium macroreticulatum* from the Mesoproterozoic Ruyang Group, China. (a) light microphotograph showing specimen with numerous regularly spaced cylindrical processes that flare outward; (b-c, e-f) SEM images showing (b) whole specimen, with inset showing details of process morphology, (c) outer wall surface covered with ridges that delimit granular polygonal fields, (e) wall reticulation, and (f) inner wall surface of closely packed, beveled hexagonal plates; (d) TEM image showing the two appressed walls of a single specimen – note multilayered wall comprising a thick electron-dense homogeneous layer of organic plates (ii) between an outer layer of debris and processes – note base of process at bottom left of center (iii) and a thin electron-tenuous layer (i) that lines the inner side of plates. Scale bar in a = 57 μm for a, 50 μm for b (20 μm for inset), 1.2 μm for c and e, 0.5 μm for d, and 2.5 μm for f.

Figure 2. Diversity of late Paleoproterozoic to early Mesoproterozoic eukaryotic fossils. (a) *Tappania plana*, from the early Mesoproterozoic Roper Group, Australia; (b) *Horodyskia moniliformis*, from the Mesoproterozoic Bangemall Group, Western Australia; (c,f) *Satka favosa*, from the Roper Group, (c) showing the wall construction of hexagonal plates, shown under SEM in (f); (d,e) *Valeria lophopstriata*, showing ornamentation of closely spaced parallel ridges on the inner wall surface in SEM (d) and light microscopic (e) view; (g,h) *Leiosphaeridia* sp., an unornamented spheroidal acritarch, with a complex wall composed of two electron-dense, homogeneous layers (i) that sandwich a thick central layer with electron-dense, porous texture (ii) visible in TEM cross-section (h); *Grypania spiralis*, a coiled

macrofossil compression from the Mesoproterozoic Gaoyuzhuang Formation, China (courtesy of M. Walter). Scale bar = 40 μm for (a), 7.8 mm for (b), 35 μm for (c), 4μm for (d), 15 μm for (e), 7.5 μm for f, 1 μm for (h), and 3 mm for i.

Figure 3. Late Mesoproterozoic and Neoproterozoic eukaryotic fossils: (a,b) "*Tappania plana*" from the Neoproterozoic Wynniatt Formation, arctic Canada, a complex form with septate, anastomosing processes, shown in detail in (b); (c) *Bangiomorpha pubescens*, from the late Mesoproterozoic Hunting Formation, arctic Canada, showing radial division of cells within a discrete zone of uniseriate filaments; (d) *Konglingiphyton erecta*, a macroscopic, dichotomously branched alga from the Ediacaran Doushantou Formation, China; (e) *Eosaccharomyces ramosa* from the late Mesoproterozoic Lakhanda succession, Siberia, showing net-like distribution on a bedding surface, with cells aligned along strands; (f) *Segmentothallus asperus* from the Lakhanda succession, a large uniseriate filament; (g) *Appendisphaera grandis*, a large acritarch with numerous, symmetrically arranged processes, from the Ediacaran Khamaka Formation, Siberia; (h) *Kildinosphaera verrucata*, an ornamented acritarch from the Neoproterozoic Miroyedikha Formation, Siberia; (i) *Bonniea dacruchares*, a vase-shaped protistan test from the Neoproterozoic Kwagunt Formation, Grand Canyon, U.S.A.; (j) preserved cast and mold of vase-shaped protist in silicified carbonates of the Neoproterozoic Ryssö Formation, Svalbard. Scale bar = 100 μm in (a), 12 μm in (b), 40 μm in (c) 4 mm in (d) 150 μm in (e), 500 μm in (f), 70 μm in (g), 25 μm in (h), 43 μm in (i), and 75 μm in (j).

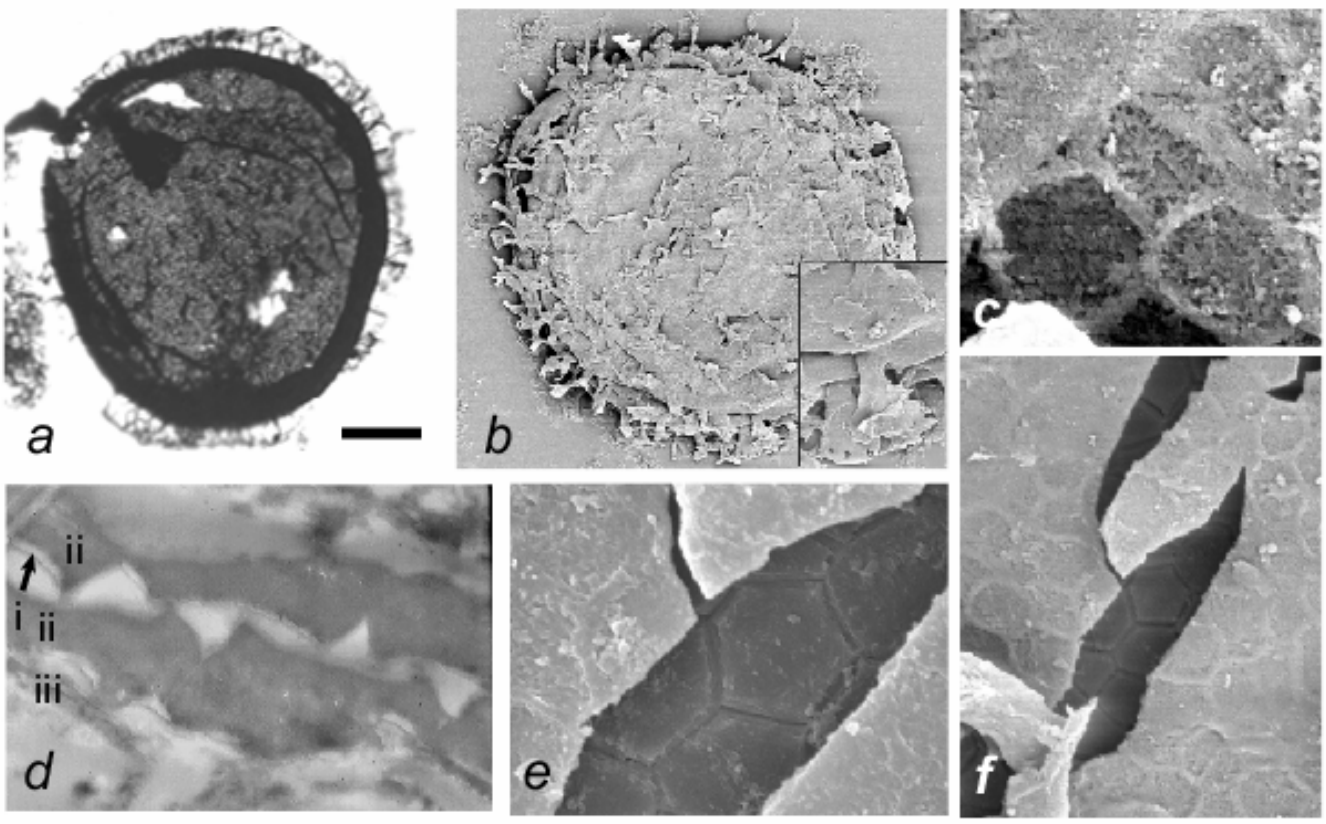
Figure 4. The composition and taxonomic richness of non-metazoan eukaryotes in Proterozoic to Early Cambrian fossil assemblages. (a) Total diversity of eukaryotic morphospecies for selected Proterozoic and Early Cambrian assemblages – in each column, a thin partition separates acritarchs from non-acritarchous protists; see legend in figure for compositions. Numbers refer to individual assemblages (principal references in parentheses; note that diversity estimates in the figure are the present authors' and do not in every case coincide with estimates in the primary references): (1) Changcheng Gr. (Yan & Liu 1993), (2) Sarda Fm. (Prasad & Asher 2001), (3) Avadh Fm. (Prasad & Asher 2001), (4) Ruyang Gr. (Xiao et al. 1997; Yin 1997), (5) Roper Gr. (Javaux et al. 2001, 2003, 2004), (6) Chamberlin Fm. (Horodyski 1982), (7) Hunting Fm. (Butterfield 2000, 2001) ; (8) Dundas Gr. (Samuelsson et al. 1999), (9) Changlongshan Fm.(Du & Tian 1985) ; (10) Lakhanda Fm. (Jankauskas 1989; Herman 1990), (11) Miroyedikha Fm. (Jankauskas 1989; Herman 1990), (12) Lower Visingsö Gr. (Vidal 1976), (13) Båtsfjord Fm. (Vidal & Siedlecka 1983), (14) Upper Visingsö Gr. (Vidal 1976), (15) Wynniatt Fm. (Butterfield & Rainbird 1998; Butterfield 2005a,b); (16) Svanbergfjellet Fm. (Butterfield et al. 1994; Butterfield 2004); (17) Chuar Gr. (Vidal & Ford 1985; Porter et al. 2003); (18) Ungoolya Gr. (Grey 2005); (19) Doushantuo Fm. (Yuan et al. 2002); (20) Pertatataka Fm. (Zang & Walter 1992); (21) Yuryakh Fm. (Moczydlowska et al. 1993); (22) Lantian Fm. (Yuan et al. 2002); (23) Redkino Gr. (Burzin et al. 1997); (24) Lower Nama Gr. (Germs et al. 1986); (25) Vergale Horizon, Baltic drillcore (Volkova et al. 1983); (26) Radzyń and Kaplonosy Fms., lower part (Moczydlowska 1991); (27) Radzyń and Kaplonosy Fms., upper part (Moczydlowska 1991); (28) Baltic Depression drillcore, assemblage 1 (Hagenfelt 1989); (29) Baltic Depression drillcore, assemblage 2 (Hagenfelt 1989); Læså Fm. (Moczydlowska & Vidal 1992); (30) Fucoid Beds (Downie 1982);

Tokammane Fm. (Knoll & Swett 1987). (b, c, and d) show the taxonomic richness of assemblages through time for acritarchs (b), macrofossil compressions (c), and (d) multicellular microfossils and vase-shaped protists; width of rectangles indicates permissible age range for assemblages; C with arrow indicates position of Proterozoic-Cambrian boundary.

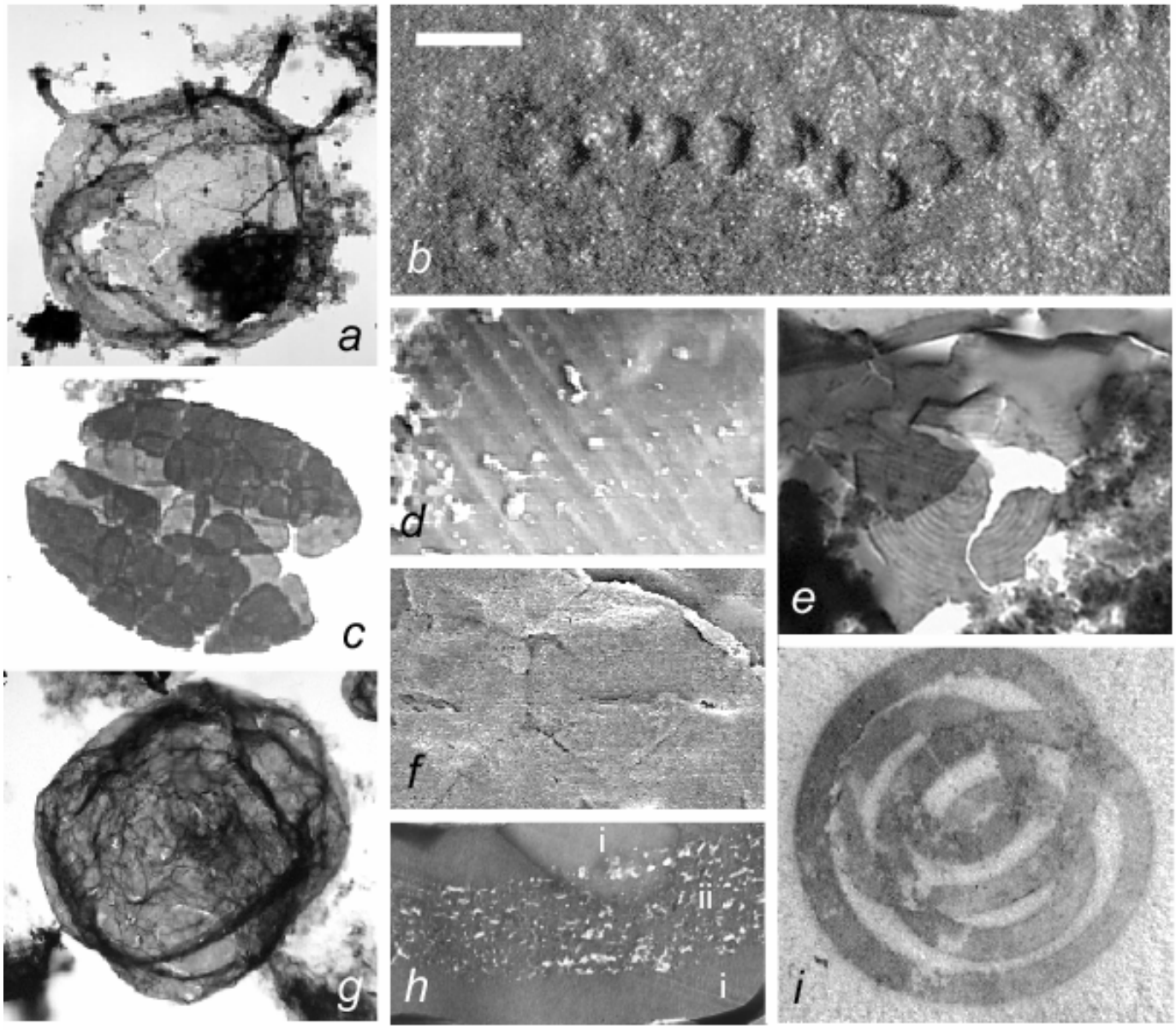
Appendix

Table providing estimates of taxonomic richness used to construct **Figure 4.** (Note that numbers provided here reflect the present authors' diversity estimates, which do not necessarily coincide with those in original publications.) **Age max** and **min** = maximum and minimum age constraints on units (Ma); **Acr U** = unornamented spheroidal acritarchs; **Acr O** = ornamented spheroidal acritarchs; **Acr AP** = acritarchs with asymmetrically arranged processes; **Acr SP** = acritarchs with symmetrically arranged processes; **VSMs** = vase-shaped microfossils; **MM** = microscopic multicellular eukaryotes; **Macro** = macroscopic protists; **Total** = total protistan diversity within the stratigraphic unit.

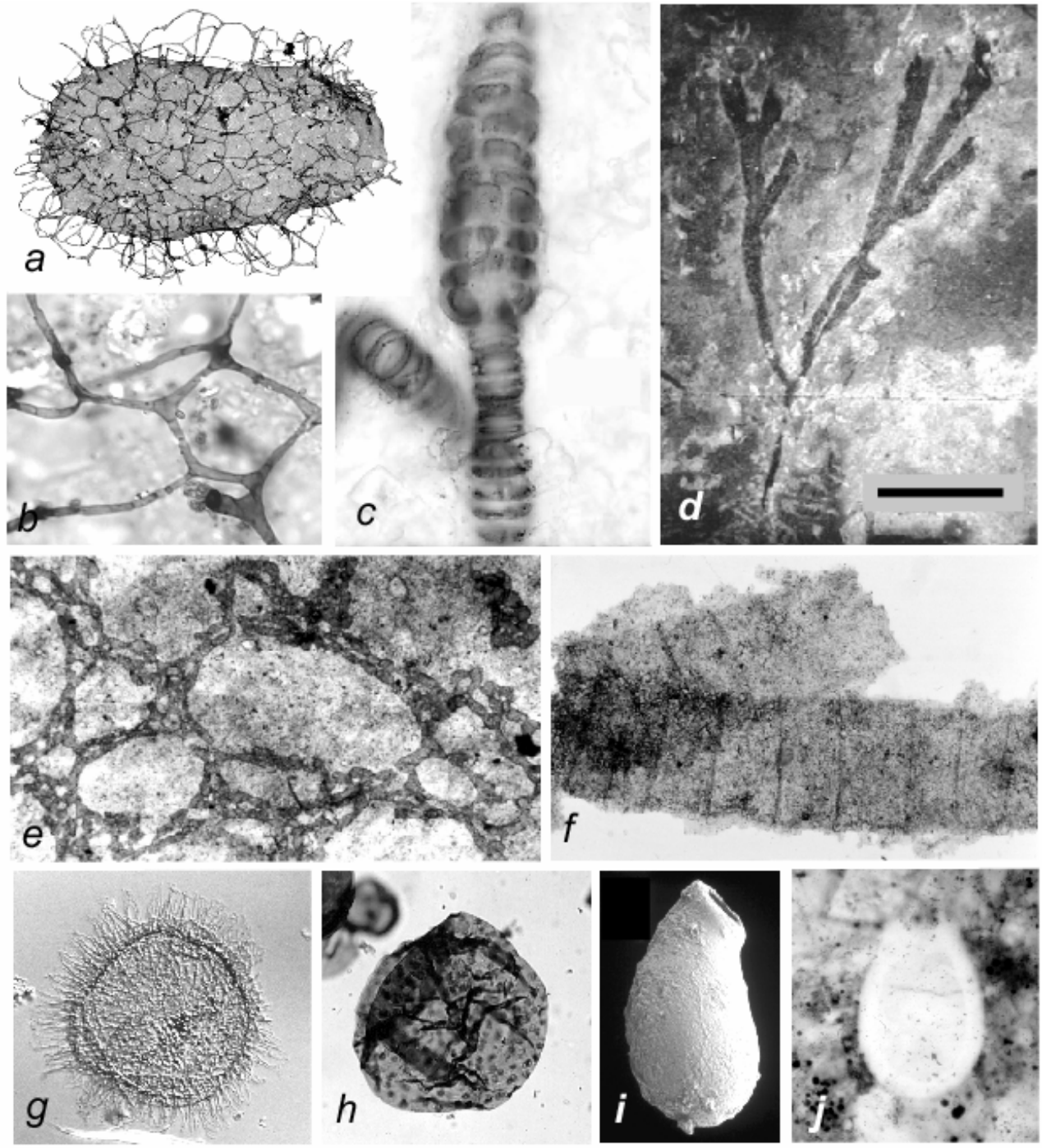
Stratigraphic Unit (# in Fig. 4a)	Location	Age max (Ma)	Age min (Ma)	Acr U U	Acr O	Acr AP	Acr SP	VSMs	MM	Macro	Total	Principal References
1. Changcheng Gr	China	1800	1600	2	1	1	0	0	0	0	4	Yan & Liu 1993
2. Sarda Fm	India	1600	1000	3	0	1	0	0	0	0	4	Prasad & Asher 2001)
3. Avadh Fm	India	1600	1000	3	1	1	0	0	0	0	5	Prasad & Asher 2001)
4. Ruyang Gr	China	1600	1250	2	1	1	1	0	0	0	5	Xiao et al. 1997; Yin 1997
5. Roper Gr	Australia	1500	1450	4	3	1	0	0	0	0	8	Javaux et al 2001, 2003, 2004
6. Chamberlain Fm	USA	1470	1425	1	0	0	0	0	0	2	3	Horodyski 1982
7. Hunting Fm	Canada	1300	1200	2	0	0	0	0	1	0	3	Butterfield 2000, 2001
8. Dundas Gr	Greenland	1300	1200	2	7	0	1	0	0	0	10	Samuelsson et al. 1999
9. Changlongshan Form	China	900	850	0	0	0	0	0	0	3	3	Du & Tian 1985
10. Lakhanda Gr	Russia	1100	1005	3	2	3	0	0	5	0	13	Jankauskas 1989, German 1990
11. Miroyedikha Fm	Russia	1000	800	4	3	1	0	0	2	0	10	Jankauskas 1989, German 1990
12. Visingö Gr (Lower, Mid)	Sweden	900	840	4	3	0	0	0	0	0	7	Vidal 1976
13. Båtsfjord Fm	Norway	900	800	2	5	0	1	0	0	0	8	Vidal & Siedlecka 1983
14. Visingö Gr (Upper)	Sweden	840	800	4	6	0	1	1	0	0	12	Vidal 1976
15. Wynniatt Fm	Canada	850	750	3	5	2	4	0	5	2	21	Butterfield & Rainbird 1994; Butterfield 2005a,b
16. Svanbergfjellet Fm	Norway	800	735	6	5	3	4	0	5	2	25	Butterfield et al. 1994; Butterfield 2004
17. Chuar Gr	USA	780	750	3	5	0	2	13	1	0	24	Vidal & Ford 1985; Porter et al. 2003
18. Ungoolya Gr	Australia	632	560	3	4	3	23	0	0	4	37	Grey 2005
19. Doushantuo Fm	China	632	551	2	5	0	24	0	11	20	62	Yuan et al. 2002
20. Pertatataka Fm	Australia	632	550	3	3	0	19	0		0	25	Zang & Walter 1992
21. Yuryakh Fm	Russia	632	550	2	0	0	9	0	0	0	11	Moczydlowska et al. 1993
22. Lantian Fm	China	560	550	0	0	0	0	0	0	7	7	Yuan et al. 2002
23. Redkino Suite	Russia	555	543	4	1	1	0	0	0	4	10	Burzin et al. 1997
24. Nama Gr	Namibia	550	543	2	0	1	0	0	0	0	3	Germs et al. 1986
25. Vergale Horizon	Estonia	525	510	3	20	1	18	0	0	0	42	Volkova et al. 1983
26. L. Radzyń/Kaplonosy Fms.	Poland	525	510	1	16	0	11	0	0	0	28	Moczydlowska 1991
27. U. Radzyń/Kaplonosy Fms.	Poland	525	510	1	13	1	18	0	0	0	33	Moczydlowska 1991
28. Baltic depression ass. 1	Sweden	525	510	3	20	0	14	0	0	0	37	Hagenfelt 1989
29. Baltic depression ass. 2	Sweden	525	510	5	24	0	16	0	0	0	45	Hagenfelt 1989
30. Laeså Fm.	Denmark	525	510	1	17	0	12	0	0	0	30	Moczydlowska & Vidal 1992
31. Fucoid Beds	Scotland	525	510	2	18	1	14	0	0	0	35	Downie 1982
32. Tokammane Fm.	Norway	525	510	2	15	0	15	0	0	0	32	Knoll & Swett 1987



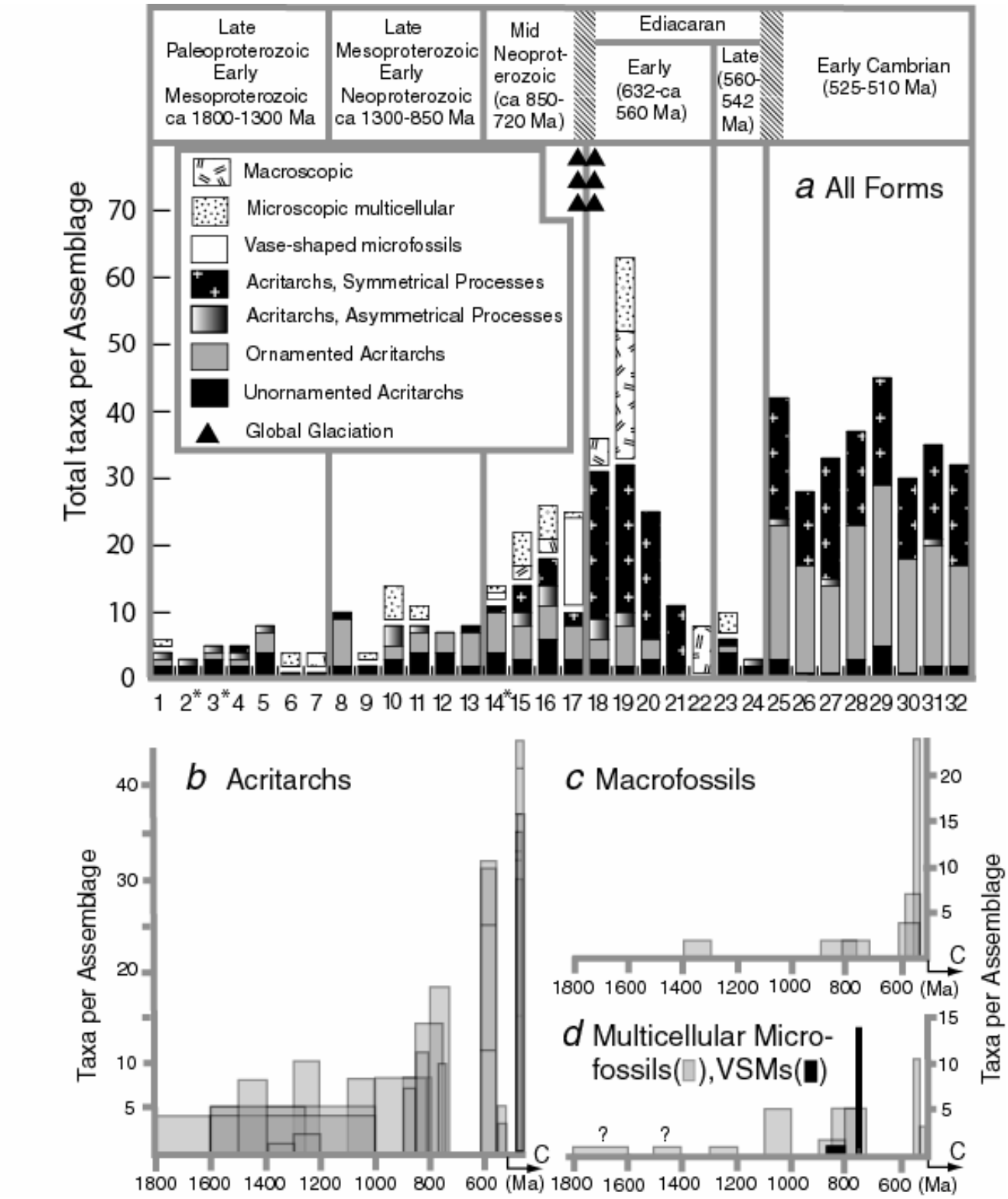
Knoll et al Fig. 1



Knoll et al. Fig. 2



Knoll et al. Fig. 3



Knoll et al. Fig. 4